REMARKS

Status of Claims

Claims 1, 3 and 6-27 are pending. Claims 14-27 have been withdrawn from consideration. Claims 1, 3 and 6-13 have been rejected.

Claim 11 has been voluntarily amended for clerical purposes only. This amendment does not narrow the scope of the claim, nor is it being made for reasons of patentability.

Claims 8 and 13 have been canceled without prejudice or disclaimer. In making this cancellation without prejudice, Applicants reserve all rights in these claims to file divisional and/or continuation patent applications.

Objections to the Drawings

The Examiner pointed out that the drawings fail to comply with 37 CFR 1.84(p)(5) because they include the following reference character(s) not mentioned in the description: Figure 2 (M1 and M2).

Applicants assert that Fig. 2 has been amended to match the specification. The entire drawing sheet containing each corrected drawing is enclosed for review by the Examiner.

Objections to the Specification

The Examiner pointed out that trademark terminology should be capitalized and accompanied by the generic terminology in the application. Applicants have made the appropriate corrections. The trademark terminology TO-PRO-3 has been amended to the generic trademark terminology TO-PRO®-3. Applicants assert that the amendments to the specification are editorial in nature and do not introduce new matter.

Claim Objections

In the Office Action, the Examiner objected to claim 13 as allegedly being of improper dependent form for failing to further limit the subject matter of a previous claim. Claim 13 has been canceled, rendering the objection moot. Accordingly, Applicants request withdrawal of the objection.

CLAIM REJECTIONS

35 U.S.C. § 112 Rejections

In the Office Action, the Examiner rejected claims 1, 6 and 8 under 35 U.S.C. § 112, first paragraph, as allegedly failing to comply with the enablement requirement.

The Examiner alleged that claim 8 is dependent upon the method of claims 1 and 6 which requires staining a PBL population obtained from a subject with an intracellular protein stain, wherein said intracellular protein stain comprises CFSE and since there was no detectable difference between control CD8 levels and beryllium-sensitized individuals, it would be impossible to determine beryllium sensitivity in a subject using the instant method and the cell surface marker CD8. specification states that there may be a significant difference in response of CD3 and CD3/CD4 T-cells from beryllium-sensitized subjects, and that no difference were noted in the response of CD8 T-cells from either normal donors or beryllium sensitized individuals consistent with previous reports.

Applicants disagree. The specification provides shows that cells from the beryllium-sensitive population had a significant positive response to 10 μM $BeSO_4$ in the CD3+ population and to 10 μM and 100 μM $BeSO_4$ in the CD3+/CD4+ population thereby providing sufficient enablement for determining beryllium sensitivity in a subject using the cell surface markers CD3 and CD4 (see paragraphs 0096 and 0098 of the published specification).

Further, to expedite prosecution, Claim 8 has been canceled, rendering the rejection moot. Accordingly, Applicants request the withdrawal of the claim rejections.

35 U.S.C. § 103 Rejections

In the Office Action, the Examiner rejected claims 1, 3 and 6, 7, 9-13 under 35 U.S.C. § 103(a), as allegedly being unpatentable over Fontenot et al. (*The Journal of Clinical Investigation*. 112(5). 2003). ("Fontenot").

The Examiner alleged that Fontenot teaches a method wherein peripheral blood mononuclear cells (PBMCs) and bronchoalveolar lavage (BAL) cells from subjects diagnosed with chronic beryllium disease (CBD) are stained with monoclonal antibodies to CD4, CD8 and CD28 in order to identify the lymphocyte (T-cell) population and contacting the identified BAL T-cell subpopulation with the intracellular protein strain CFSE.

Applicants respectfully disagree for the reasons set forth below. The claimed invention is directed to "determining beryllium sensitivity…comprising staining a **peripheral blood leukocyte (PBL)**…with…CFSE." [emphasis added]. Fontenot does not teach or suggest this claimed feature. Rather, Fontenot relates to labeling branchoalveolar lavage (BAL) CD4+ T cells with CFSE, where they even performed the analysis on separated (by cell sorting) CD4+ BAL T cells.

Biologic responses on separated cell populations frequently differ from those in their native, unseparated environment where factors from other cell types can influence response. Thus, the response of the separated CD4+ T cells in the lung (BAL) may differ significantly from CD4+ T cells evaluated in the context of other components of the peripheral blood, due to the microenvironment of the cells, unexpectedly rendering the latter to be a more robust and relevant diagnostic biomarker.

The Examiner acknowledges that Fontenot does not teach "a method wherein PBL is used with CFSE in a beryllium sensitivity assay." However, the Examiner, without any factual data or support, merely asserts that one can substitute PBL T-cells for the Fontenot's BAL cells. Further, Fontenot's purpose was to determine how the function of the BAL cells was impaired by decreased CD28. Indeed Fontenot states that:

> Here, we examined the role of CD28-mediated costimulation in antigen-specific T cell activation and survival. The results demonstrate an apparent evolution of independence from CD28-mediated costimulation that correlates with memory cell differentiation. Memory $CD4^+$ T cells in blood continued to require CD28 costimulation for proliferative and cytokine responses to beryllium. In the lung, proliferation and secretion of Th1-type cytokines by effector memory cells were functionally independent of CD28 costimulation, and a proportion of these cells stopped expressing CD28. These $CD4^+CD28^-$ T cells showed decreased proliferative capacity and an increased rate of apoptosis after stimulation with antigen, suggesting transition to a presenescent state.

(See, Fontenot et al. p. 777, top-left paragraph). Hence, Fontenot's purpose was not to determine if there was a predictive response of PBMC to beryllium salt (as measured by CFSE) as a disease indicator, as demonstrated by the present invention (see paragraphs 0096-0099).

In addition, Fontenot did not use CD3 in the sort criteria, which is a marker of T cells, instead they used CD4 and CD28 (see Methods, p. 777, left-hand column, 3rd paragraph), therefore the cells they were measuring included monocytes as well and were not necessarily T cells at all. Thus, a skilled artisan would not be able to readily use Fontenot in a predictive manner to arrive at the present invention.

In summary, Fontenot et al. did NOT use CFSE to measure the proliferation of $CD3^+/CD4^+$ peripheral T cells and thus did not address the differences in this response between normal subjects and subjects exposed to beryllium.

In view of the foregoing amendments and remarks, Applicants assert that the pending claims are allowable. Their favorable reconsideration and allowance is respectfully requested.

Should the Examiner have any question or comment as to the form, content or entry of this Amendment, the Examiner is requested to contact the undersigned at the telephone number below. Similarly, if there are any further issues yet to be resolved to advance the prosecution of this application to issue, the Examiner is requested to telephone the undersigned counsel.

Please charge any fees associated with this response to deposit account No. 50-3355.

Respectfully submitted,

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